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Molecular Epidemiology of Adenovirus Type 7 in Israel: Identification of Two New Genome Types, Ad7k and Ad7d2

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The molecular epidemiology of Adenovirus type 7 in Israel was investigated. Fifty-seven adenovirus isolates identified as serotypes 7 or 7a which were recovered from patients in Israel between 1968 and 1995 were analyzed by restriction enzymes digestion using BamHI for primary discrimination and identification of genome types and by six additional enzymes: BstEII, HpaI, BglI, BglII, BclI, and XbaI for confirmation and determination of genomic subtypes. Four digestion patterns were identified with BamHI; one of them was new. Using BstEII, two patterns were obtained, one of them new. Digestion with the other five enzymes yielded known patterns. The analysis revealed four different genomic types and subtypes, which circulated in Israel in different years: subtype 7a1; type 7b, a type with a new BamHI pattern which was designated type 7K, and a subtype with a new BstEII pattern which differed from type 7d by one restriction site and was designated type 7d2. Twenty-two isolates from 1968 through 1975 and from 1984 were Ad7a1. Three isolates from 1973–1974 were Ad7b. Five isolates from 1968 through 1973 were Ad7K and 27 isolates from 1992 through 1995 were Ad7d2. This demonstrates the temporal change in the circulating genome types with up to three genome types cocirculating in 1 year (1973). The two new types, Ad7k and Ad7d2 could have evolved in Israel or could have been imported by travellers and immigrants from neighboring or distant countries. ***J. Med. Virol. 54:291–299, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: Adeno 7, restriction enzymes; molecular typing

INTRODUCTION

Adenovirus type 7 has been frequently associated with outbreaks of systemic infections with severe clinical symptoms, such as pneumonia, gastroenteritis, ocular infections with high fever, and central nervous system symptoms [Horwitz, 1996; Sharp and Wadell, 1994; Simila et al., 1971].

This serotype displays an extensive genetic variablility when molecular analysis, using restriction enzyme digestion with various enzymes is employed [Li et al., 1996].

So far, 13 genomic types and 22 subtypes have been idenfitifed according to the classification intitially described by Wadell et al. [1985] and reassessed by Li and Wadell [1986] and by Li et al. [1996]. According to their system, BamHI is used for basic discrimination between genomic types. This enzyme can distinguish between 13 genome types of Ad7. Additional enzymes, including BstEII, BclI, EcoRI, HindIII, HpaI, XbaI, BglI, and BglII, can be used to further distinguish between subtypes of the 13 types. Over the years various research groups have been using this system to analyse Ad7 genomic types thereby describing the global molecular epidemiology of this virus [Li et al., 1996; Li and Wadell, 1986; Wadell et al., 1985; De Silva et al., 1989; Golovina et al., 1991; Niel et al., 1991; Kajon and Wadell, 1992a,b, 1994; Kajon et al., 1996; Kannemeyer et al., 1988; Adrian et al., 1989]. Various genome types and subtypes circulated in different countries and continents in different years and caused outbreaks with various degrees of severity. No direct link was established between a particular type and enhanced virulence. It seems that the immunity of the population plays a major role in determination of the extent of the outbreak and severity of symptoms. Some genome types were found only in one continent: Ad7h, Ad7i, and Ad7j have so far been found only in South America [Kajon et al., 1996]. A similar situation existed for Ad7a which was initially found only in China, but was later detected also in the US. Ad7g is still endemic to China, but other genomic types such as 7b, 7c, and 7e

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TABLE I. Identification Process of Ad7 Genome Types and Subtypes: Initial Selection of Four Genome Types by Bam HI Digestion and Progressive Identification of Subgenomic Types Using Six Additional Enzymes

Additional restriction enzymes	BamHI digestion patterns and types			
	I a1 ~ 6	II New-derived from a1 ~ 6	III b, b1	IV d, d1
BstEII	NAI[a]	NAI	b	New-derived from d
HpaI	a1 ~ 5	Derived from a1 ~ 5	NAI	NAI
Bg1I	a1 ~ 3, 5	Derived from a1 ~ 3, 5	NAI	NAI
Bg1II	a1, 3, 5	Derived from a1, 3,	NAI	NAI
Bc1I	a1, 3	Derived from a1, 3	NAI	NAI
XbaI	a1	Derived from a1	NAI	NAI
Conclusion	a1	New ~ k	b	New ~ d2

[a]NAI, No additional information.

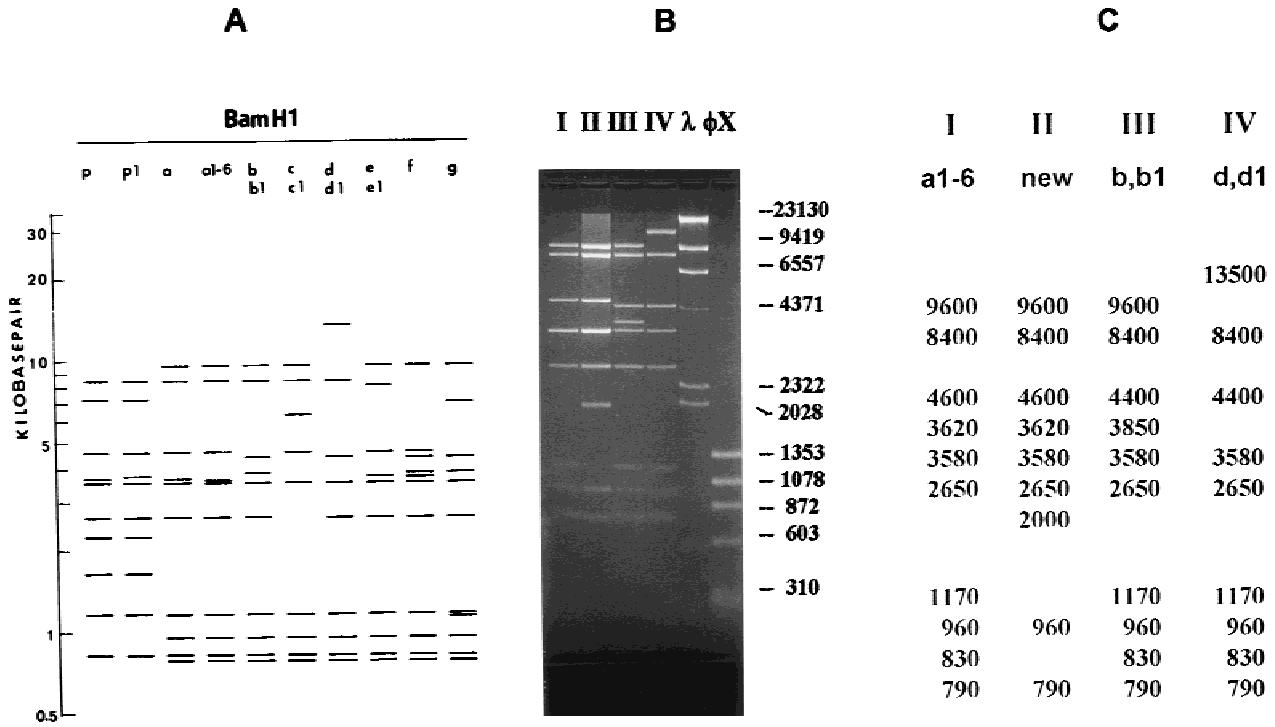

Fig. 1. Restriction patterns of BamHI. **A:** Restriction patterns diagram published by Li et al. Journal of Medical Virology 49: 170–172, 1996; **B:** BamHI Restriction pattern of our isolates resolved by electrophoresis on agarose gel and ethidium bromide staining. One representative for each pattern (I, II, III, and IV) is shown as indicated at the top of the gel. λHindIII and ϕ× HaeIII markers, which ran on the same gel, are indicated at the top and their fragments sizes are indicated at the right hand side of the gel; **C:** Calculated sizes of fragments of each pattern (calculation was done as described in "Materials and Methods") and pattern identification according to the diagram shown in A.

are widespread in all five continents. Newly discovered genomic subtypes identified by enzymes other than BamHI are more exclusive to their country or continent of origin such as Ad7a2 and Ad7a6 in the USA, Ad7a4 in China, and Ad7c1 in South Africa. However, it seems that with time the new types spread to new places. Adenovirus type 7 epidemiology in the Middle East has so far not been described.

Israel has a small population but a substantial percent of it consists of new immigrants and frequent travellers. Thus, we expected to find several genomic types which circulated over the years in the Israeli population. Surprisingly, we found only four genomic types among our isolates which circulated in Israel between 1968 and 1995.

MATERIALS AND METHODS

Virus Isolation

Clinical samples were submitted to our laboratory for viral diagnosis from hospitals and clinics in the central part of Israel. Virus was isolated according to standard procedures on human kidney cells (Huki), a line of epithelial cells established in our laboratory and used

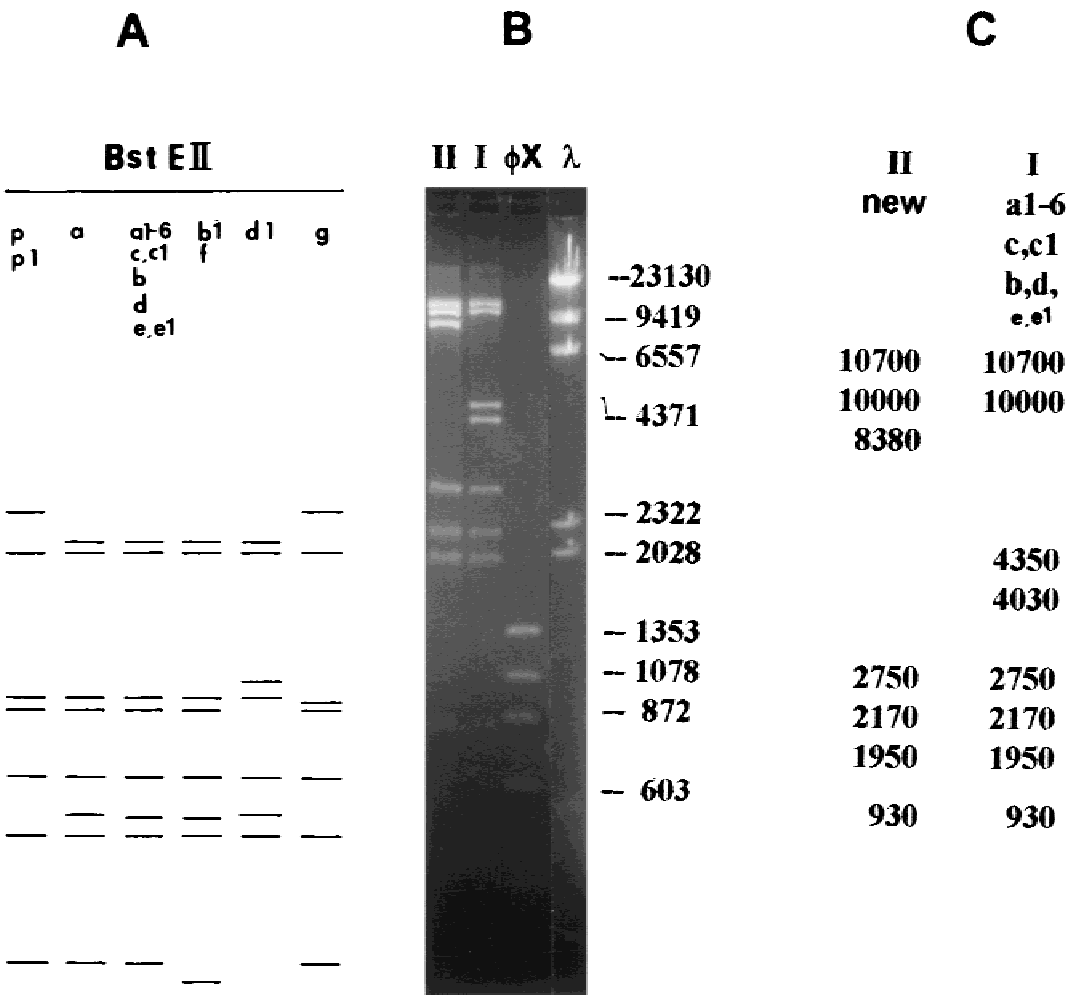

Fig. 2. Restriction patterns of BstEII. **A:** Restriction patterns diagram published by Li et al. Journal of Medical Virology 49: 170–177, 1996; **B:** BstEII restriction pattern of our isolates resolved by electrophoresis on agarose gel and ethidium bromide staining. One representative for each pattern (I and II) is shown as indicated at the top of the gel. ϕ× HaeIII and λHindIII markers, which ran on the same gel, are indicated at the top and their fragments sizes are shown at the right hand side of the gel; **C:** Calculated sizes of fragments of each pattern (calculation was done as described in "Materials and Methods") and pattern identification according to the diagram shown in A.

for routine diagnosis. Following the appearance of cytopathic effect (CPE) cultures were frozen and used as viral stocks. Virus isolates were kept at –20°C.

Virus Identification

Identification of virus type was done by neutralization assays, using rabbit anti adenovirus type 7 and 7a antisera. The sera were obtained between 1962 and 1963 from the Department of Health Education and Wellfare, Public Health Services, Communicable Diseases Center, Biological Reagents Section, Atlanta, GA, or prepared in our laboratory using isolates which were identified using the above mentioned antisera.

Preparation of Viral DNA

Virus removed from –20°C was innoculated onto A549 cell monolayers (ATCC-CCL185) in a T-75 flask with Eagle's MEM-NAA containing 0.3 mg/ml glutamine, 25 u/ml mycostatine, 0.6 mg/ml Streptomycin, 600 u/ml penicilin, and supplemented with 5% heat inactivated Fetal Calf Serum (FCS). Cells were incubated at 37°C with 5% CO_2. Following the appearance of Cytopathic Effect (CPE) viral DNA was prepared by a modification of Hirt procedure [Tratschin et al., 1984] except that exctraction of DNA included a phenol-chloroform step. Following the last precipitation with EtOH, DNA was resuspended in 100 μl of TE buffer and stored at –20°C. Extraction, precipitation, and RNase treatment were done as described before [Sambrook et al., 1989].

Restriction Enzyme Analysis of Viral DNA and Gel Electrophoresis

Aliquots of 2–5 μg viral DNA were digested at 37°C for 5–17 hr with one of the following enzymes: BamHI, BstEII, BglII, BglI, HpaI, BclI, and XbaI with 5–10 unit/μg DNA. The enzymes and digestion buffers were from New-England Biolabs, Beverly, MA. Two to 5 μg of the digested DNA were electrophoresed on 1–1.5% agarose (Seakem, LE FMC) gels in Tris-Acetate buffer

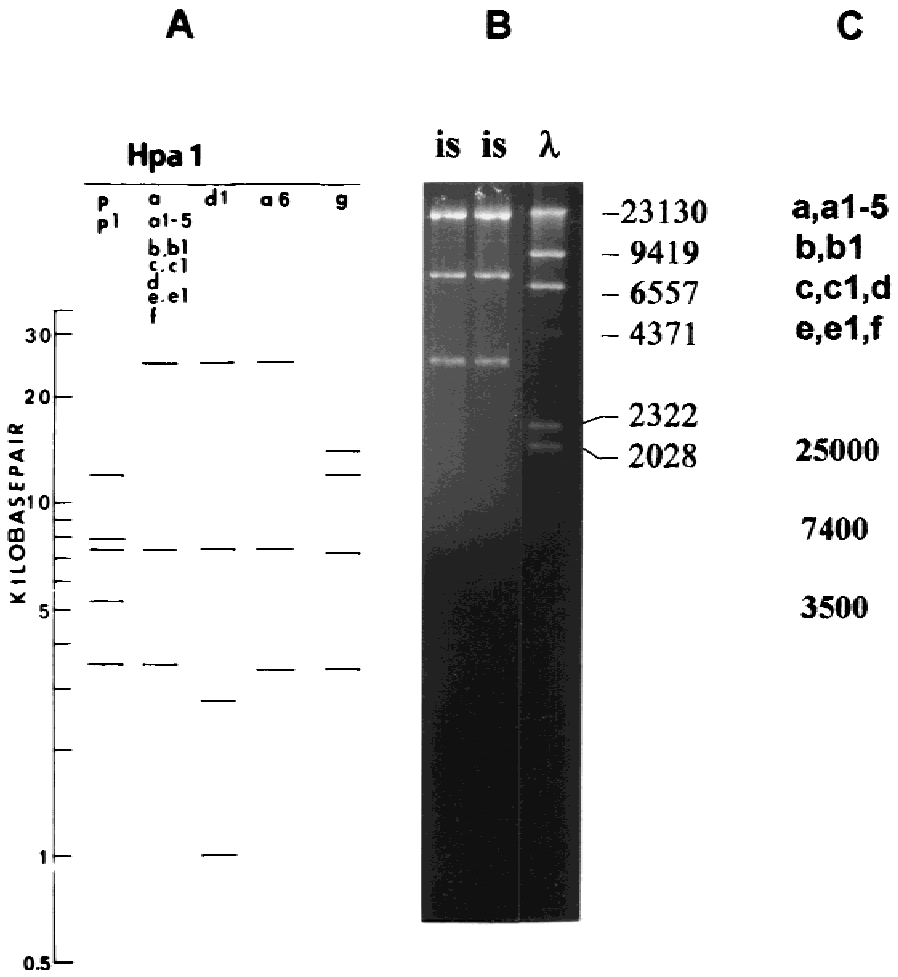

Fig. 3. HpaI Restriction patterns. **A:** Restriction patterns diagram published by Li et al. Journal of Medical Virology 49: 170–177, 1996; **B:** HpaI restriction enzyme pattern of two representatives of our isolates (is) and λHindIII markers resolved by electrophoresis on agarose gel and ethidium bromide staining. The sizes of the λHindIII fragments, which ran on the same gel, are indicated at the right hand side of the gel; **C:** Calculated sizes of the isolate's HpaI fragments (calculation was done as described in "Materials and Methods") and pattern identification according to the diagram shown in A.

[Sambrook et al., 1989] containing 0.5 μg/ml Ethidium-Bromide for 16 hr at 1.5 V/cm. The gels were visualized and photographed under U.V. light.

Measurement of Restriction Fragment Sizes

λDNA digested with HindIII, ϕ×174 DNA digested with HaeIII, both from New-England Biolabs, and a 123 base-pair DNA ladder, BRL, Paisley, Scotland, were electrophoresed alongside with the adenovirus DNA. A plot of the log of the marker fragments sizes VS migration distances in mm was prepared separately for four different ranges of size: 23000–9500, 9500–4500, 4500–2000, and 2000–600. The sizes of the adenovirus fragments were determined by interpolation and by comparison to the previously recognized patterns [Li and Wadell, 1986; Wadell et al., 1985]. When a new fragment appeared its exact size was determined according to our measurement taking into account the known size(s) of the original fragments if a new restriction site was involved or a restriction site was lost.

RESULTS

Adenovirus Types 7 and 7a Isolates Between 1968 and 1995

Fifty-seven clinical isolates which we had serologically identified as types 7 or 7a, as described in Materials and Methods and which were stored at –20°C, were available for analysis. They were associated with various clinical symptoms, including fever (49%), congestion (37%), pneumonia (21%), vomiting (4%), and myocarditis (4%): 6% of them were fatal cases.

Frozen isolates were thawed and propagated on A549 cells and viral DNA was prepared from each isolate, as described in Materials and Methods.

Identification of Genome Types by Restriction Enzyme Analysis

We used the approach initially described by Wadell et al. [1985] with additional modifications according to Li and Wadell [1986] and Li et al. [1996]. Although we did not use the entire panel of restriction enzymes de-

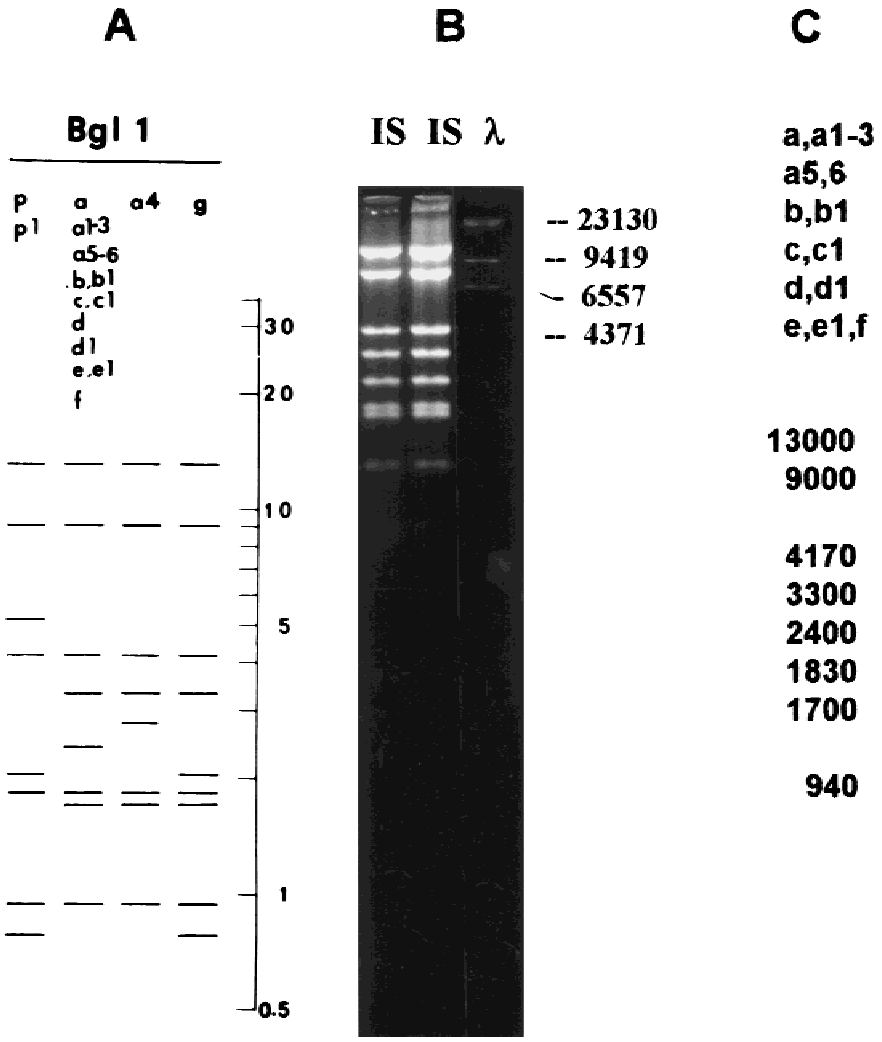

Fig. 4. BglI Restriction patterns. **A:** Restriction patterns diagram published by Li et al. Journal of Medical Virology 49: 170–177, 1996; **B:** BglI restriction enzyme pattern of two representatives our isolates (IS) and λHindIII marker fragments resolved by electrophoresis on agarose gel and ethidium bromide staining. The sizes of the λHindIII fragments, which ran on the same gel, are indicated at the right hand side of the gel; **C:** Calculated sizes of the isolate's BglI fragments (calculation was done as described in "Materials and Methods") and pattern identification according to the diagram shown in A.

scribed in these three papers, we followed the general scheme by using BamHI as the major discriminating enzyme for classification, then used other restriction enzymes BstEII, BglII, BglI, HpaI, BclI, and XbaI for confirmation, and further distinction of the subgenome types. All our isolates were characterized as outlined bellow and summarized in Table I.

Digestion with BamHI revealed four different patterns (I–IV) shown in Figure 1B. The sizes of fragments were determined (Fig. 1C) and compared to previousely known patterns (Fig. 1A). Three of them (patterns I, III, and IV) were identical to previously described genome-types [Li and Wadell, 1986; Li et al., 1996] corresponding to genome types: a1–6 (pattern I); b,b1 (pattern III), and d,d1 (pattern IV).

Pattern number II was new and did not correspond to any of the previously described patterns. This pattern is directly derived from pattern I (a1–6) by the loss of one restriction site which forms the seventh and ninth fragments of a1–6 (Fig. 1c). These two fragments with lengths of 1,170 bp and 830 bp disappear and a new 2,000 bp long fragment appears (seventh from top in pattern II). There were 22 isolates with pattern I, five isolates with pattern II, three isolates with pattern III, and 27 isolates with pattern IV.

Digestion with BstEII yielded only two patterns: I and II (Fig. 2B): the first one, corresponding to genome types a1–6, b, c, c1, d, e, e1 (Fig. 2A,C) was found among isolates with BamHI patterns I, II, and III leading to the identification of BamHI pattern III as type b rather than b1 (Table I). The second BstEII pattern is derived from the first one by loss of one restriction site which forms the third (4,350 bp) and fourth (4,030 bp) fragments of pattern I (Fig. 2c) yielding a new 8,380 bp fragment (third from top in pattern II). The new pattern was found only among isolates with BamHI pattern IV (d, d1) identifying them as a new subgenomic type derived from genomic type d [Table I].

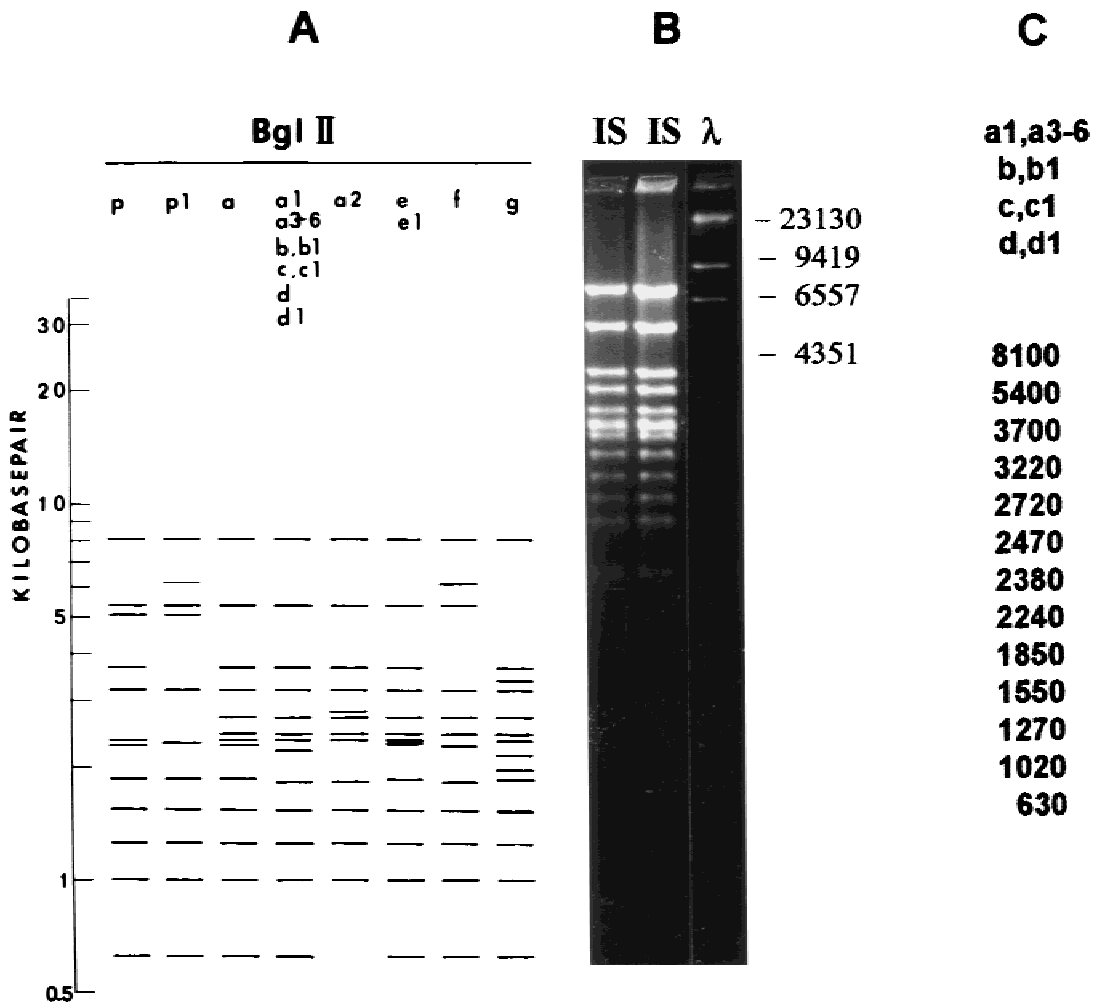

Fig. 5. BglII Restriction patterns. **A:** Restriction patterns diagram published by Li et al. Journal of Medical Virology 49: 170–177, 1996; **B:** BglII restriction enzyme pattern of two representatives of our isolates (is) and λHindIII markers resolved by electrophoresis on agarose gel and ethidium bromide staining. The sizes of the λHindIII fragments, which ran on the same gel, are indicated at the right hand side of the gel; **C:** Calculated sizes of the isolate's BglII fragments (calculation was done as described in "Materials and Methods") and pattern identification according to the diagram shown in A.

Digestions with HpaI, BglI, BglII, BclI, and XbaI were performed on representative isoaltes of the four Bam HI and the two BstEII patterns. All isolates displayed identical patterns which excluded out genome types d1 and a6 (HpaI); a4 (BglI); a2 (BglII); a5 (BclI); and a3 (XbaI) (Figs. 3–7).

After excluding genome types a, a2–6, d, d1,e, f, and g, we examined the new BamHI and BstEII patterns for possible identity to recently described genome types h, i, and j [Kajon et al., 1996] and c1 and e1 [Li et al., 1996]. No such identity was found.

In summary, we found four different combinations among our isolates: 1) BamHI pattern I/BstEII pattern I: which corresponds to genome type a1. 2) BamHI pattern II/BstEII pattern I: a combination which corresponds to a new genome type by the BamHI pattern and was therefore designated Ad7k according to the system described by Li et al. [1996]. 3) BamHI pattern III/BstEII pattern I: which corresponds to genome type b. 4) BamHI pattern IV/BstEII pattern II: which corresponds to a new genomic subtype derived from genome type d according to Li et al. [1996] and was designated Ad7d2.

Yearly Distribution of the Four Genome Types

The four genome types of adenovirus type 7 which were identified: Adeno 7a1, Adeno 7b, Adeno 7k, and Adeno 7d2 cirulated in Israel at different periods. Table II shows the temporal distributon of these genome-types between 1968 and 1995.

Adenotype 7a1 circulated continuousely between 1968 and 1975 and was found again in 1984. The new genome type Ad7k which differs from Ad7a1 by one restriction site with BamHI cocirculated with Ad7a1 in 1968, 1969, 1971, and 1973. It was not found later among the isolates which were available for molecular analysis. Genome type 7b was found only in 1973 and 1974, cocirculating with types 7k and 7a1. The second new genome subtype Ad7d2 was first detected in 1992.

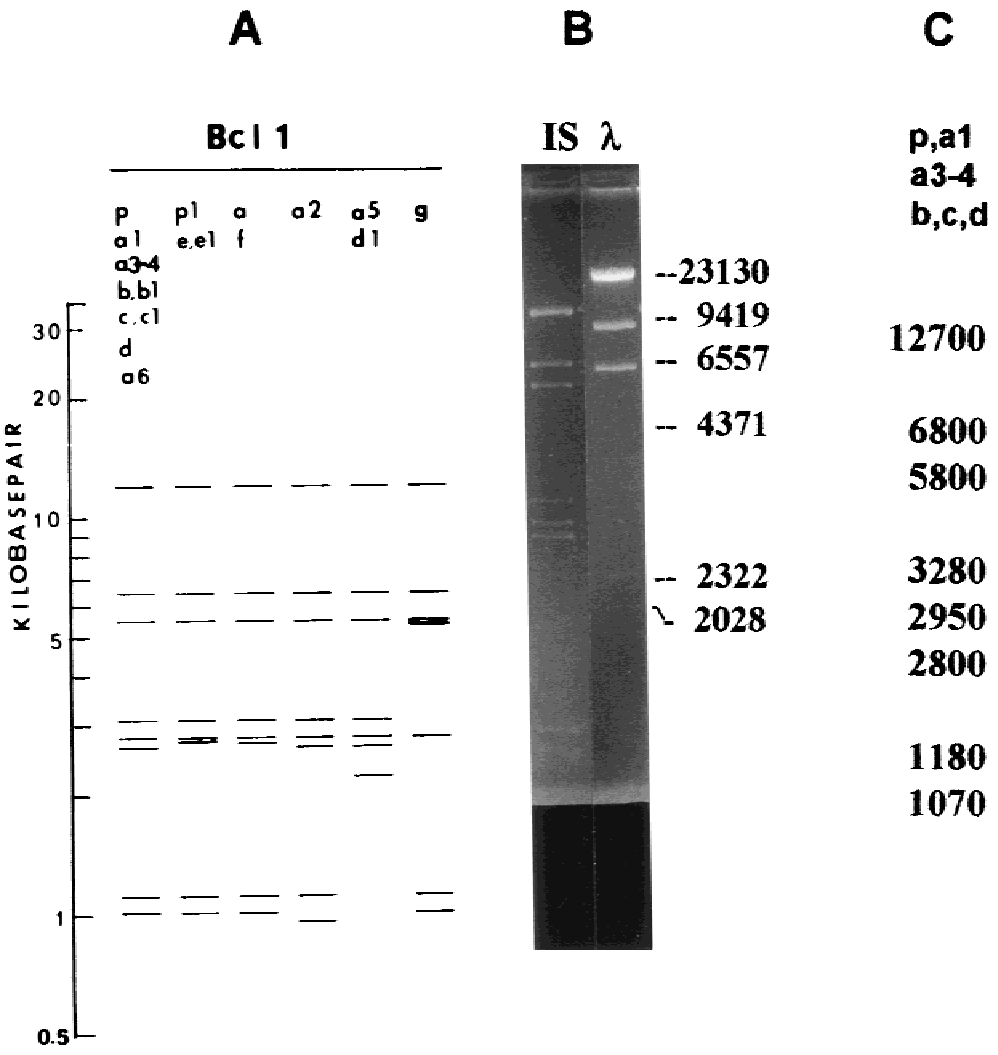

Fig. 6. Bcl1 Restriction patterns. **A:** Restriction patterns diagram published by Li et al. Journal of Medical Virology 49:170–177, 1996; **B:** BclI restriction enzyme pattern of one representative of our isolates (IS) and λHindIII markers resolved by electrophoresis on agarose gel and ethidium bromide staining. The sizes of the λHindIII fragments, which ran on the same gel, are indicated at the right hand side of the gel; **C:** Calculated sizes of the isolate's Bcl1 fragments (calculation was done as described in "Materials and Methods") and pattern identification according to the diagram shown in A.

It has continuosely circulated since then and was the only Ad7 genome type which has been isolated in our laboratory since 1992. There were additional isolates serologically identified as type 7a, which were not available for molecular analysis in 1981(3), 1983(2), 1984(1), 1985(1), 1988(1), and 1990(1).

DISCUSSION

The Central Virology Laboratory provides clinical diagnosis to the central region of Israel, in which the majority of the population resides and investigates outbreaks.

We have analyzed 57 isolates of adenovirus, serotyped as Ad7 or Ad7a by following the system initially described by Wadell et al. [1985] and Li and Wadell [1986] with modifications as suggested by Li et al. [1996]. This system was followed because it has been used by various research groups around the world allowing a comparison of our isolates to genome types which circulated in other parts of the world.

We found four different genomic types which circulated between 1968 and 1995 in Israel. Two of them have been described before: Ad7a1 and Ad7b. Two others are new types: Ad7k and Ad7d2. The four genome types circulated at different years with temporal order and two of them, Ad7a1 and Ad7d2 predominated among our isolates. No large outbreak of any adenovirus type 7 was recorded during these years.

Of a particular interest is the fact, that Ad7b and Ad7d2 isolates were initially identified serologically as Ad7a1 even though they are not directly derived from Ad7a.

The clinical symptoms associated with our adeno type 7 isolates included mostly lower respiratory tract symptoms severe enough to require hospitalization. However, cases with mild symptoms could occur in the

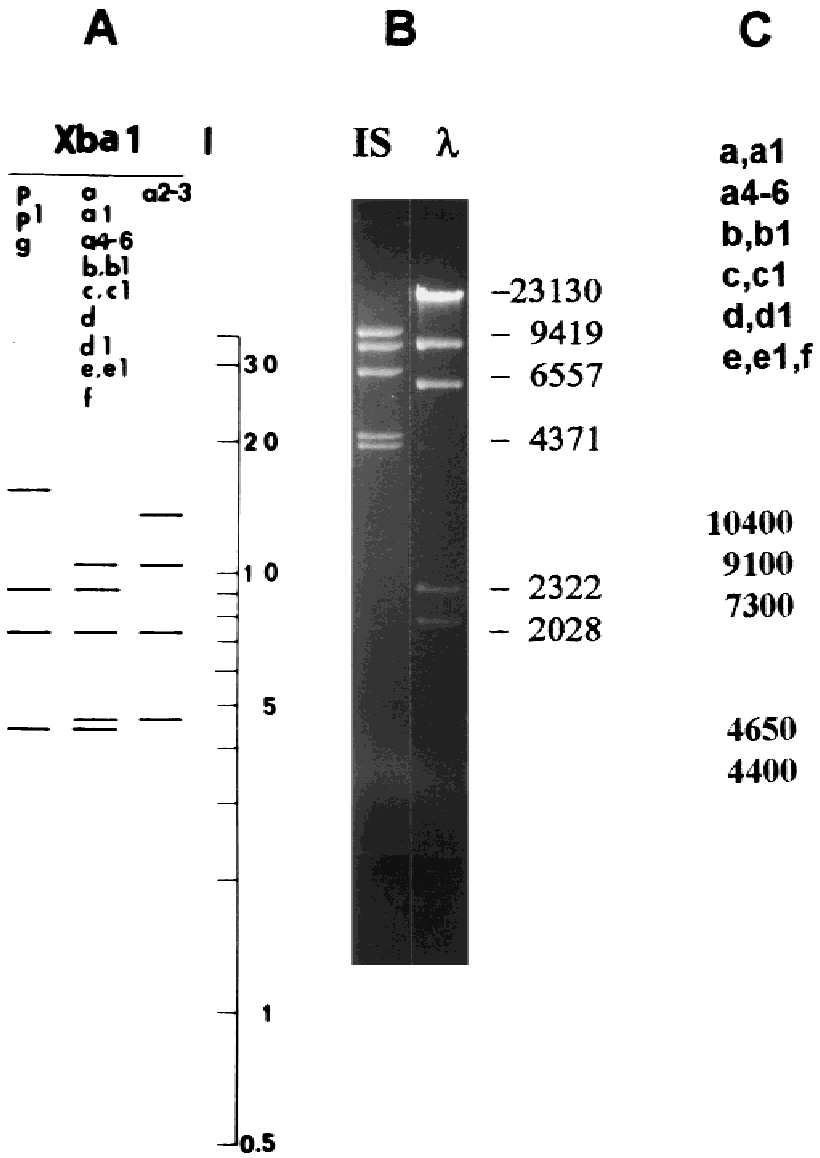

Fig. 7. XbaI Restriction patterns. **A:** Restriction patterns diagram published by Li et al. Journal of Medical Virology 49:170–177, 1996; **B:** Restriction enzyme pattern of one representative of our isolates (is) and λHindIII markers resolved by electrophoresis on agarose gel and ethidium bromide staining. The sizes of the λHindIII fragments, which ran on the same gel, are indicated at the right hand side of the gel; **C:** Calculated sizes of the isolate's XbaI fragments (calculation was done as described in "Materials and Methods") and pattern identification according to the diagram shown in A.

TABLE II. Yearly Distribution of Ad7 Genome Types in Israel Between 1968 and 1995

Year	68	69	70	71	72	73	74	75	84	92	93	94	95	Total
Genome type	Number of Isolates													
Ad7a1	3	5	1	2	4	2	2	1	2	0	0	0	0	22
Ad7k	1	1	0	2	0	1	0	0	0	0	0	0	0	5
Ad7b	0	0	0	0	0	1	2	0	0	0	0	0	0	3
Ad7d2	0	0	0	0	0	0	0	0	0	4	7	11	5	27
Total	4	6	1	4	4	4	4	1	2	4	7	11	5	57

community for which samples have never been sent to the laboratory for diagnosis.

The new type Ad7k can be directly derived from Ad7a1 which circulated here and could therefore have evolved in Israel. In contrast, the other new type, Ad7d2, is derived directly from Ad7d for which we have no evidence of circulation in Israel, and possibly could be imported from another country or continent in which it has not yet been detected. In particular, it could have been imported from the former USSR by the

immigrants who came in large numbers during the early 1990's or from Ethiopia by another group of immigrants in 1992.

The global distribution of adenovirus type 7 genome types was assessed with reference to our isolates [Li et al., 1996; Li and Wadell, 1986; Wadell et al., 1985; De Silva et al., 1989; Golovina et al., 1991; Niel et al., 1991; Kajon and Wadell, 1992a,b, 1994; Kajon et al., 1996; Kannemeyer et al., 1988; Adrian et al., 1989]. Adeno type 7b is the most widely spread virus: it has been found in Africa, Australia, Europe, US, China, UK, and South America. It was found among our isolates only in 1973–1974. In contrast, Adeno type 7a1 which was found only in China and Australia, circulated in Israel for many years. We did not identify adeno type 7C which is also widely spread (Africa, Europe, Sweden, US, and South America).

Adeno 7k and Adeno 7d2 could be endemic to our region, however, no data is available at present from other countries in this region.

ACKNOWLEGMENT

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